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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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5 Applicants : Christoph Reinhard, Anne B. Jefferson, Jill A. Winter, and
Filippo Randazzo
Serial No. : 09/875,440
Filed : January 5, 2001
10 For : COMPOSITIONS AND METHODS FOR TREATING
NEOPLASTIC DISEASE USING NET-4 MODULATORS

Examiner : Sean R. McGarry
Art Unit : 1635
Docket No. : PP-01701.002/59516-149
Date : June 20, 2003

15 Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

AFFIDAVIT OF DR. A. B. JEFFERSON UNDER 37 C.F.R. § 1.132
(IN SUPPORT OF RESPONSE UNDER 37 C.F.R. § 1.112)

(0-510-7-3)
Sir:

I, Dr. A. B. Jefferson, being duly sworn, say:

20 1. I am a true and original inventor of the claimed subject matter of the above-
25 identified patent application.

2. I am an internationally recognized scientist and am presently employed as
Principal Scientist at Chiron Corporation, Emeryville, California (from 1996 to present). I
received a Bachelors Degree in Biology from University of Richmond and a Ph.D. degree from
Stanford University in Pharmacology.

30 3. I am an author or co-author of 19 peer-reviewed research articles and have been
invited to give numerous presentations on my research at national and international meetings.
My curriculum vitae is attached as Exhibit 1.

4. In my capacity as Principal Scientist, I am familiar with methods of inhibiting cell
growth and of evaluating the biological effects of antisense oligonucleotides, antibodies, and

other mechanisms of inhibiting cell growth, such as cancer cell growth, including methods well-known to those of ordinary skill in the art at the time of filing of the above-identified patent application.

5 5. I understand that claims of the above-referenced patent application are rejected under 35 U.S.C. § 101, based on alleged lack of patentable utility, and also under 35 U.S.C. § 112, first paragraph, on the grounds that one skilled in the art would not know how to use the claimed invention, because the claimed invention is allegedly not supported by a patentable utility. I generally understand that patentable utility refers to either a well documented utility, or a specific, substantial, and real world utility.

10 6. The application as filed shows that inhibition of NET-4 with antisense oligonucleotides specific for NET-4 inhibits the growth of colon cancer cells. These data are disclosed in Example 2 at pages 33-35, and Example 4 at pages 39-40. The specification also discloses that the expression level of NET-4 in colon tumor cells is at least *2-fold greater* than that of matched normal colon cells, as described in Example 5 at pages 40-43. In addition, the
15 *in situ* hybridization studies of NET-4 expression in normal colon and lung tissues, as compared with colon tumor and lung tumor tissues, indicate *increased expression in the tumor tissues* (see Example 6 at page 43, and Figure 2).

20 7. Information available in the scientific literature that I have reviewed indicates that inhibition using antisense correlates with inhibition of the same protein using antibodies. These proteins are discussed below

25 8. **CD44.** Naor *et al.* reported the inhibition of CD44 expression in tumor cells. CD44 was targeted using *antibodies* and *antisense oligonucleotides*, and the targeting resulted in reduction of the malignant activities of the tumor cells. (Naor *et al.*, *Crit Rev. Clin Lab.*, 39:527-579, 2002.) For example, at page 554, Naor describes studies in which treatment of animals with anti-CD44 antibodies suppressed a variety of malignant activities. At page 555, Naor states, “[d]ownregulation of tumor-supporting CD44 by specific antisense transfection *is an alternative way of proving that CD44 (especially CD44v) targeting is a rational approach to cancer therapy.*” (Emphasis added.) Although the results were, in part, specific to the biological role of CD44, the overall finding was a reduction in tumor spread.

9. **EGFR.** Pomerantz *et al.* reported that blocking of epidermal growth factor receptor (EGFR) with monoclonal antibodies, and with antisense oligonucleotides, is being investigated for anti-cancer therapy, in view of the upregulation of EGFR in many types of human tumors. (Pomerantz *et al.*, *Curr. Oncol. Rep.* 5:140-146, 2003.) Pomerantz reported that antisense oligodeoxynucleotides targeting the translation start sites of EGFR inhibited the proliferation of head and neck squamous carcinoma cells (HNSCC). In other studies, antisense constructs directed against EGFR inhibited tumor growth when administered intratumorally to HNSCC xenografts in nude mice. These authors also reported that monoclonal antibodies specific for EGFR inhibited the growth of HNSCC cell lines, and these studies led to human studies of anti-EGFR antibody therapy of HNSCC.

10. **VEGF.** Another protein family that is important in normal and tumor cell growth, vascular endothelial growth factor, or VEGF, contains as a member VEGF-C, which is implicated in malignant mesothelioma growth. Masood *et al.* found that antisense oligonucleotide complementary to VEGF inhibited VEGF expression and also specifically inhibited mesothelioma cell growth. Antibodies to VEGF receptor also inhibited mesothelioma cell growth. Although in this case the antibodies and antisense were directed to different proteins, the two proteins are functionally related (protein and its receptor), indicating that the ultimate effect was to prevent the protein from carrying out its normal biological role. (Masood *et al.*, *Int. J. Cancer* 104:603-610, 2003.)

11. **IGF.** Stearns *et al.* reported that inhibition of insulin-like growth factor (IGF) receptor using antisense specific for IGF receptor polynucleotides, and using IGF receptor-specific antibodies, had similar effects on the ability of IL-10 to block IGF activation of mRNA expression and protein synthesis in cancer cells. (Stearns *et al.*, *Clin. Cancer Res.* 9:1191-1199, 2003.)

12. It is therefore my opinion that there is a correlation between inhibition of protein expression using specific antisense oligonucleotides, and inhibition of the same protein using specific antibodies.

13. I further declare that all statements made herein of my own knowledge are true and that these statements are made with the knowledge that willful false statements and the like

so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code.

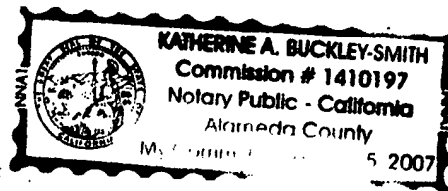
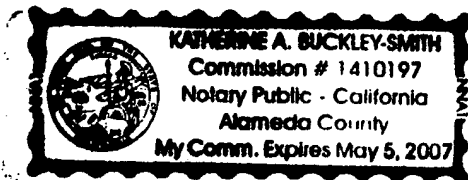
A. B. Jefferson
A. B. Jefferson

5 State of California)
County of Alameda) ss.:

10 On this 20 day of JUNE, 2003, before me, a Notary Public in and for the State and County aforesaid, personally appeared A. B. Jefferson, to me known and known to me to be the person of that name, who signed and sealed the foregoing instrument, and ~~SHE~~ acknowledged the same to be her free act and deed.

15 Katherine A. Buckley-Smith
Notary Public

Commission expires May 5, 2007



Anne Bennett Jefferson

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Date of Birth: January 18, 1961
Place of Birth: Farmville, Virginia

Education:

- 1983 B. S. Biology (honors), University of Richmond, Virginia
B. A. History, University of Richmond, Virginia
- 1990 Ph. D. Stanford University School of Medicine, Department of
Pharmacology (Advisor: Howard Schulman)

Professional Positions:

- 1989-1992 Postdoctoral Fellow, Division of Hematology, Washington University
School of Medicine (Advisor: Philip W. Majerus)
- 1992-1996 Research Associate, Division of Hematology, Washington
University School of Medicine
- 1996-1999 Scientist I, Research, Chiron Technologies, Chiron Corporation
- 1999-2001 Scientist II, Research, Chiron Technologies, Chiron Corporation
- 2001-present Principal Scientist, Chiron Technologies, Chiron Corporation

Academic and Professional Awards:

- Dickinson Undergraduate Research Award, 1982
- Pharmaceutical Manufacturer's Association Predoctoral Fellowship, 1987-1988
- Frances Lou Kallman Award for excellence in science and graduate study
Stanford University, 1989

Invited Lectures:

- University of Richmond, Department of Biology, January 1994

Refereed Publications:

- Schulman, H., Kuret, J., Jefferson, A.B., Nose, P. S., and Spitzer, K. H. (1985) Ca^{2+} /calmodulin-dependent microtubule-associated protein 2 kinase: broad substrate specificity and multifunction potential in diverse tissues. *BIOCHEM.* 24: 5320-5327.
- Jefferson, A. B. and Schulman, H. (1988) Sphingosine inhibits calmodulin-dependent enzymes. *J. BIOL. CHEM.* 263: 15241-15244.
- MacNicol, M., Jefferson, A. B., and Schulman, H. (1990) Ca^{2+} / calmodulin kinase is activated by the phosphatidylinositol signaling pathway and becomes Ca^{2+} independent in PC12 cells. *J. BIOL. CHEM.* 265: 18055-18058.
- Mitchell, C. A., Jefferson, A. B., Bejeck, B. E., Brugge, J. E., Deuel, T. E., and Majerus, P. W. (1990) Thrombin-stimulated immunoprecipitation of phosphatidylinositol 3-kinase from human platelets. *PROC. NATL. ACAD. SCI.* 87: 9396-9400.
- Majerus, P. W., Ross, T. S., Cunningham, T. W., Caldwell, K. K., Jefferson, A. B., and Bansal, V. S. (1990) Recent insights in phosphatidylinositol signaling. *CELL* 63: 459-465.
- Jefferson, A. B. and Schulman, H. (1991) Phosphorylation of microtubule-associated protein-2 in GH3 cells: Regulation by cAMP and by calcium. *J. BIOL. CHEM.* 266: 346-354.
- Jefferson, A. B., Travis, S. M., and Schulman, H. (1991) Activation of multifunctional Ca^{2+} / calmodulin-dependent protein kinase in GH3 cells. *J. BIOL. CHEM.* 266: 1484-1490.
- Ross, T. S., Jefferson, A. B., Mitchell, C. A., and Majerus, P. W. (1991) Cloning and expression of human 75-kDa inositolpolyphosphate-5-phosphatase. *J. BIOL. CHEM.* 266: 20283-20289.
- Jefferson, A. B. and Majerus, P. W. (1995) Properties of type II inositol polyphosphate 5-phosphatase. *J. BIOL. CHEM.* 270: 9370-9377.
- Zhang, X., Jefferson, A. B., Auethavekiat, V., and Majerus, P. W. (1995) The protein deficient in Lowe's syndrome is a phosphatidylinositol 4,5-bisphosphate 5-phosphatase. *PROC. NATL. ACAD. SCI. USA* 92: 4853-4856.
- Damen, J.E, Liu, L., Rosten, P., Humphries, R.K., Jefferson, A.B., Majerus, P.W., and Krystal, G. (1996) The 145-kDa protein induced to associate with Shc by multiple cytokines is an inositol tetrakisphosphate and phosphatidylinositol 3,4,5-trisphosphate 5-phosphatase. *PROC. NATL. ACAD. SCI. USA.* 93: 1689-1693.

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Jefferson, A. B. and Majerus, P. W. , (1996) Mutation of the conserved domains of two inositol polyphosphate 5-phosphatases. *BIOCHEM.* 35: 7890-7894.

Liu, L., Jefferson, A. B., Zhang, X., Norris, F. A., Majerus, P. W., and Krystal, G. (1996) A novel phosphatidylinositol 3,4,5 trisphosphate 5-phosphatase associates with the interleukin-3 receptor. *J. BIOL. CHEM.* 271: 29729-29733.

Jefferson, A. B., Auethavekiat, V., Pot, D. A., Williams, L. T., and Majerus, P. W. (1997) Signaling inositol polyphosphate 5-phosphatase: characterization of activity and effect of GRB2 association. *J. BIOL. CHEM.* 272: 5983-5988.

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Jefferson, A. B., Klippel, A., and Williams, L. T. (1998) Inhibition of mSOS-activity by binding of phosphatidylinositol 4,5-P₂ to the mSOS pleckstrin homology domain. *ONCOGENE* 16: 2303-2310.

Zundel, W., Schindle, C., Haas-Kogan D., Koong, A., Kaper, F., Chen, E., Gottschalk, A.R., Ryan H., E., Johnson, R. S., Jefferson, A. B., Stokoe, D., Giaccia, A.J. (2000) Loss of PTEN facilitates HIF-1-mediated gene expression. *GENES DEV.* 14: 391-6.

Yan D, Wiesmann M, Rohan M, Chan V, Jefferson AB, Guo L, Sakamoto D, Caothien RH, Fuller JH, Reinhard C, Garcia PD, Randazzo FM, Escobedo J, Fantl WJ, Williams LT. (2001) Elevated expression of axin2 and hnk2 mRNA provides evidence that Wnt/beta -catenin signaling is activated in human colon tumors. *PROC. NATL. ACAD. SCI. USA.* 98: 14973-8.